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SECURITY CLASSIFICATION OF THIS PAGE

UMENTATION PAGE

Form Approved
OMB No 0704-0188

AD-A197 260

1b RESTRICTIVE MARKINGS
N/A

3 DISTRIBUTION/AVAILABILITY OF REPORT
Distribution Unlimited

2b DECLASSIFICATION/DOWNGRADING SCHEDULE
N/A

4 PERFORMING ORGANIZATION REPORT NUMBER(S)
Virginia Commonwealth University

5 MONITORING ORGANIZATION REPORT NUMBER(S)
N/A

DTIC ELECTE JUL 14 1988 S E D

6a NAME OF PERFORMING ORGANIZATION
Virginia Commonwealth University

6b OFFICE SYMBOL (If applicable)
N/A

7a NAME OF MONITORING ORGANIZATION
Office of Naval Research

6c ADDRESS (City, State, and ZIP Code)
1101 E. Marshall Street
Box 678 MCV Station
Richmond, VA 23298-0678

7b ADDRESS (City, State, and ZIP Code)
800 N. Quincy Street
Arlington, VA 22217-5000

8a NAME OF FUNDING/SPONSORING ORGANIZATION
Office of Naval Research

8b OFFICE SYMBOL (If applicable)
ONR

9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
N00014-86-K-0496

8c ADDRESS (City, State, and ZIP Code)
800 N. Quincy Street
Arlington, VA 22217-5000

10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
61153N	RR04108		

11 TITLE (Include Security Classification)
Effects of DHEA on Host Virus Interactions.

12 PERSONAL AUTHOR(S)
Loria, Roger M. and Regelson, William

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Annual	FROM 8/1986 TO 6/1987	1988, June 28	

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			DHEA; Steroid; Coxsackievirus; Herpesvirus; Immune Regulation; Resistance; Infection; HIV; Hematology;

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

A significant protective effect of a native adrenal steroid, dehydroepiandrosterone (DHEA) was demonstrated in studies of two lethal viral infection models in mice, systemic coxsackievirus B4 and herpes simplex type 2 encephalitis. The steroid was active either by long-term feeding or by a single subcutaneous injection prior to initiation of infection. A closely related steroid, etiocholanolone, was not protective in these models. Histopathological analysis, leukocyte counts, and numbers of spleen antibody forming cells in the coxsackievirus B4 model suggests that DHEA functions by maintaining or potentiating the immune competence of mice otherwise depressed by viral infection. DHEA protection was most evident when a large inoculum of virus was given, suggesting that the protective mechanism required a "triggering action" by the virus. DHEA was not effective in genetically immunodeficient HRS/J hr/hr mice and did not demonstrate antiviral activity in vitro. (Continued on reverse)

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS

21 ABSTRACT SECURITY CLASSIFICATION
(U)

22a NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c OFFICE SYMBOL
Dr. J.A. Majde	202/696-4055	ONR

DD Form 1473, JUN 86 Previous editions are obsolete

S/N 0102-LF-014-6603

SECURITY CLASSIFICATION OF THIS PAGE

89

19. Abstract (Continue)

While the molecular basis for DHEA's effect on the immune system is not known, studies by others suggest that it may counteract the stress related immunosuppressive effects of glucocorticoids stimulated by viral infection. Because DHEA is a native steroid that has been used clinically with minimal side effects, the utility of DHEA in the therapeutic modulation of acute and chronic viral infections including acquired immune deficiency syndrome (e.g., HIV) deserves intensive study.

July 5, 1988

PROGRESS REPORT ON CONTRACT N00014-85-K0525

CONTRACTOR: VIRGINIA COMMONWEALTH UNIVERSITY, SCHOOL OF BASIC HEALTH SCIENCES.

CONTRACT TITLE: EFFECTS OF DHEA ON HOST VIRUS INTERACTIONS

START DATE: AUG 1985

RESEARCH OBJECTIVES:

To determine the role of dehydroepiandrosterone (DHEA) as an **UP MODULATOR** of the host immune response and its potential use for the treatment of virus infections.

PROGRESS (07/01/87-06/30/88)

I. We have establish that protection against acute lethal viral infections with the native steroid hormone dehydroepiandrosterone (DHEA) is practical. This protection is achieved not by a direct effect against the infectious agent but apparently by **up regulation of the host immune response** to combat the infectious process.

Our results have shown that:

[1] DHEA at concentrations of 2 - 20 μM failed to influence CVB4 replication in vitro, where immune mechanisms are not present.

[2] DHEA was ineffective in the inbred HRS/J hr/hr mouse which is genetically immunodeficient.

[3] Up-regulation of the immune response by DHEA was seen in CVB4-infected mice with regard to the number of spleen IgM and IgG AFC, Figure 4 in attached manuscript.

[4] Administration of DHEA alone was also associated with enlargement of the spleen germinal centers which suggests stimulation of the B lymphocyte dependent areas.

[5] DHEA treatment of CVB4-infected animals resulted in a reduction of the "starry sky pattern", an indicator of cell killing, which was prominent in the spleens of CVB4-infected mice not treated with DHEA.

[6] Finally, an increase in circulating mononuclear cells was observed in DHEA-treated/CVB4-infected mice which is consistent with the role of these cells in host defense against CVB4 infection as does the DHEA mediated decline in the splenic "starry sky" pattern.

II. The following full length manuscripts and abstracts are published or in press:

PUBLICATIONS

1. Regelson, W., Loria, R. and Kalimi, M.
   Hormonal Intervention: "Buffer Hormones" or "State dependency". The role of Dehydroepiandrosterone (DHEA); Thyroid Hormone, Estrogen and Hypophysectomy in Aging.
   New York Academy of Sciences 518: 260-273, 1988.

2. Loria, R.M., Inge, T.H., Cook, S., Szakal, A. and Regelson, W.
   Protection against acute lethal viral infections with the native steroid dehydroepiandrosterone (DHEA).
   J. Med. Virol 26: 1988. (in Press)

3. Following the request of the editor, this manuscript was also included In:
   Adrenal Androgens in Clinical Medicine.
   Parker L., Eds. Academic Press Nov 1988.

4. Loria, R.M., Inge, T.H., Cook, S. and Regelson, W.
   Up-Modulation of the Immune Response and resistance to Virus infection with dehydroepiandrosterone (DHEA). In: Hormones, Thermogenesis, and Obesity.
   Lardy H., Eds. Academic Press, 1988.

5. Loria, R.M., Inge, T.H. and Regelson, W.
   Up Modulation of the Host Immune Response for Anti-Viral Treatment with DHEA. Second International Conference on Antiviral Research.
   Antiviral Res. 9: 154,1988. ABSTRACT

6. Loria, R.M., Inge, T.H., Cook, S. and Regelson, W.
   Up-Modulation of the Immune Response and resistance to Virus infection with dehydroepiandrosterone (DHEA).
   Eighteenth Steenbock Symposium. Hormones, Thermogenesis, and Obesity. Univ. of Wisconsin-Madison. June 1988. ABSTRACT

7. Inge, T., Loria, R., Cook, S. and Regelson, W.
   Monocytosis and Neutrophilia as Hematological Manifestations of the Anti-Viral steroid DHEA.
   Annual Md/PhD Student Conference, Aspen Colorado, July 22-25,1988. ABSTRACT

Clearly, a considerable efforts was made within the last year to bring these results to publication.

A copy of abstract nos. 5 and of publication nos. 2 are attached to this report.

III. MECHANISMS OF ACTION OF DHEA

Presently we do not know the mechanism of immune up regulation by DHEA. However several lines of evidence indicate that its mode of action is by a different pathway than the so called normal metabolic pathway of DHEA. The following results support these conclusion:

1. Protection against lethal infections by coxsackievirus B4 with DHEA was obtained if the hormone was administered in a) the diet or by subcutaneous injection. In both cases it appears that there is a need for a prolong contact with the lymphoid system to obtain an effect. Administration of DHEA by the subcutaneous route was qualitatively and quantitatively more efficient than feeding DHEA, (see figure 2 and 3 in attached manuscript).

2. Furthermore, intraperitoneal injection of DHEA did **not** have any protective effect against virus infection. We concluded from this observation that due to the fast adsorption of DHEA when injected I.P. no up regulation of immunity was achieved.

3. This conclusion is further supported by the observation that I.P. injection of DHEAS also had no protective effect, figure 1.

4. Finally, our initial results demonstrate that in order for DHEA injected S.C. to have an antiviral effect, the hormone must be administered in a lipophilic vehicle. If injected with other vehicle i.e. saline or ethanol no effect is observed.

Experiments to determine whether DHEA has an effect on IL-2 and on the distribution of B and T lymphocytes using a fluorescent activated cell sorter are in progress.

IV. EFFECTS OF DHEA ON THE DIABETIC MUTANTS db/+ HOST.

Our previous reports (1,2) have show that the homozygote diabetic mutant mouse C57BL/KsJ db/+ prior to onset of diabetes is immunologically impaired as evident by its inability to develop neutralization antibodies when infected with a human diabetogenic CVB4. The heterozygote db/+ which does not develop spontaneously diabetes respond to CVB4 infection, but its capacity to develop neutralization antibodies was 50% of the controls. Coleman, et al (4,5) reported that feeding DHEA to diabetic mutant mice resulted in recovery and normalization. Our experiments were designed to determined whether DHEA can up regulate the immune response of the diabetic mutant host. The results are presented in figure 2.

The results demonstrate that administration of a single dose of DHEA (S.C) to db/+ mice resulted in a significant protection from coxsackievirus B4 mortality for at least 11 days. The delay in virus induced mortality by DHEA in the db mutant animal suggest that the temporary protective effect of DHEA was not obtained by an increase in the level of humoral antibodies. This conclusion is base of the fact that administration of passive antibodies is sufficient to achieve protection.

V. QUANTITATION OF DHEA

Efforts to develop a Thin Layer Chromatography procedure for the accurate and reproducible simultaneous quantitation of both DHEA and DHEAS on very small samples, i.e. 0.002 - 0.025 ml will has been in progress. Results are encouraging, as evident from the data in figure 3, [solvent system: a) Ethyl Acetate to 5 cm. b) Petroleum Ether: Ethyl Acetate: Ethanol: 15N NH_4OH at a ratio of 3:5:4:3 run up to 10 cm. dry and repeat to 19 cm] but the sensitivity is still inadequate for routine use. Additional efforts to improve this procedure are in progress.

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2. Montgomery, L.B. and Loria, R.M. Humoral Immunity in Hereditary and Overt Diabetes Mellitus. Med. Virology. 12:255-268, 1986.

3. Coleman, D.L., Leiter, E.A. and Schizer, R.W. Therapeutic effects of dehydroepiandrosterone (DHEA) in diabetic mice. Diabetologia 31:830-833,1982.

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5. Coleman, D.L., Leiter, E.H. and Applezweig, N. Therapeutic effects of dehydroepiandrosterone metabolites in diabetes mutant mice (C57BL/KsJ db/db). Endocrinologia 115:238-248,1984.

Figure 1

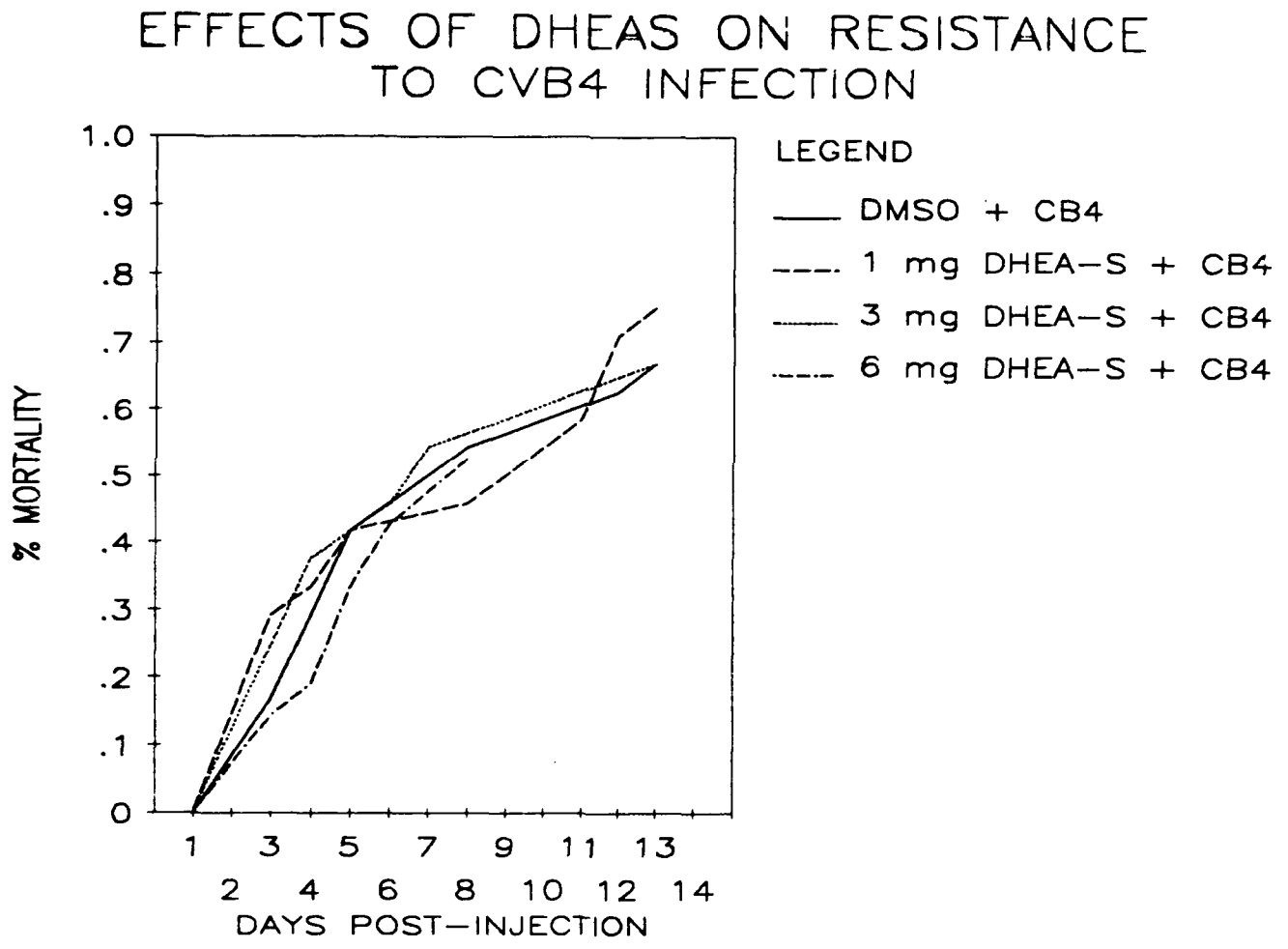
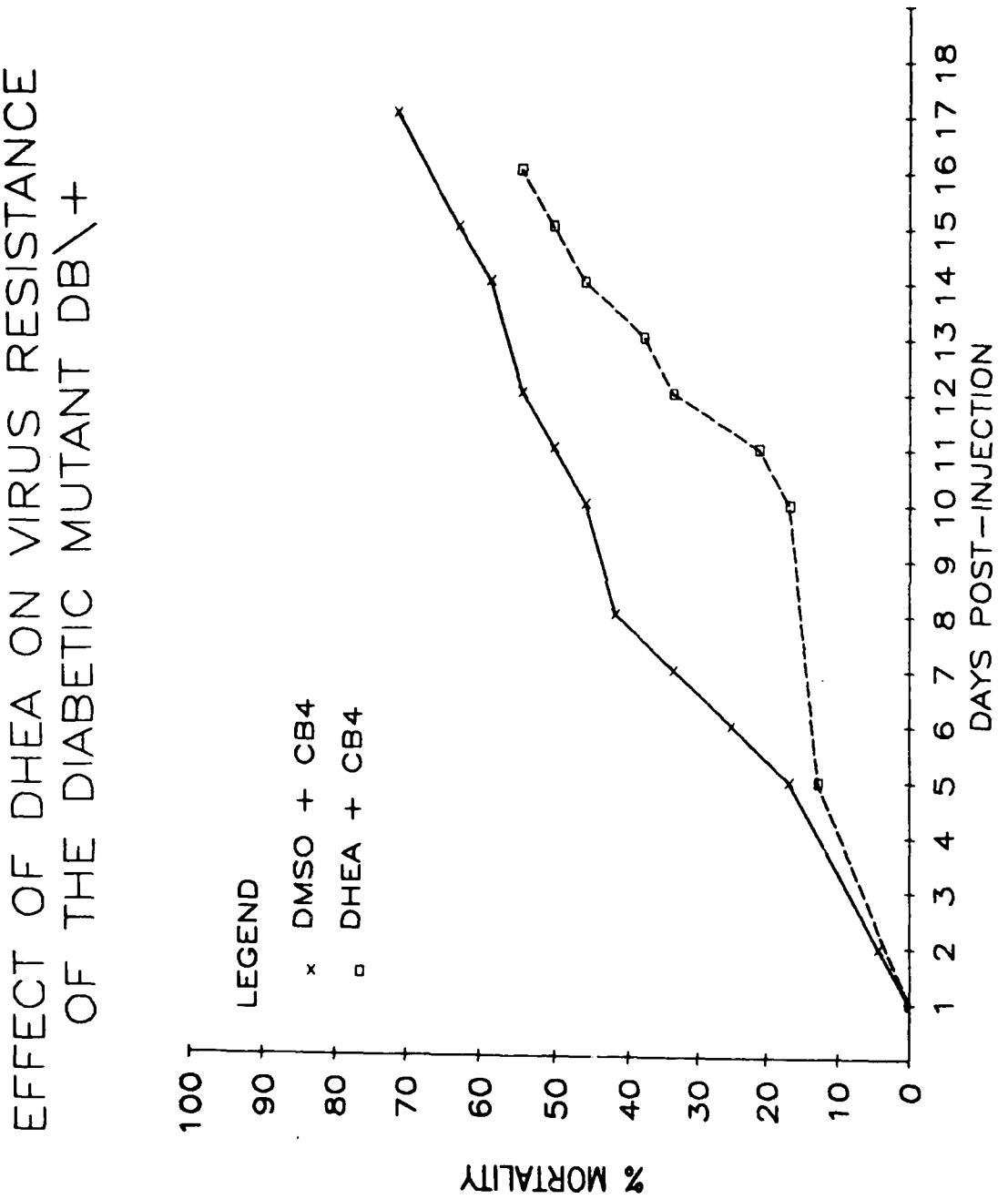

EFFECTS OF DHEAS ON RESISTANCE TO CVB4 INFECTION
% MORTALITY
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
1 3 5 7 9 11 13
2 4 6 8 10 12 14
DAYS POST—INJECTION
LEGEND
DMSO + CB4
1 mg DHEA—S + CB4
3 mg DHEA—S + CB4
6 mg DHEA—S + CB4

Figure 2


EFFECT OF DHEA ON VIRUS RESISTANCE
OF THE DIABETIC MUTANT DB\+
LEGEND
x DMSO + CB4
□ DHEA + CB4
% MORTALITY
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
DAYS POST-INJECTION

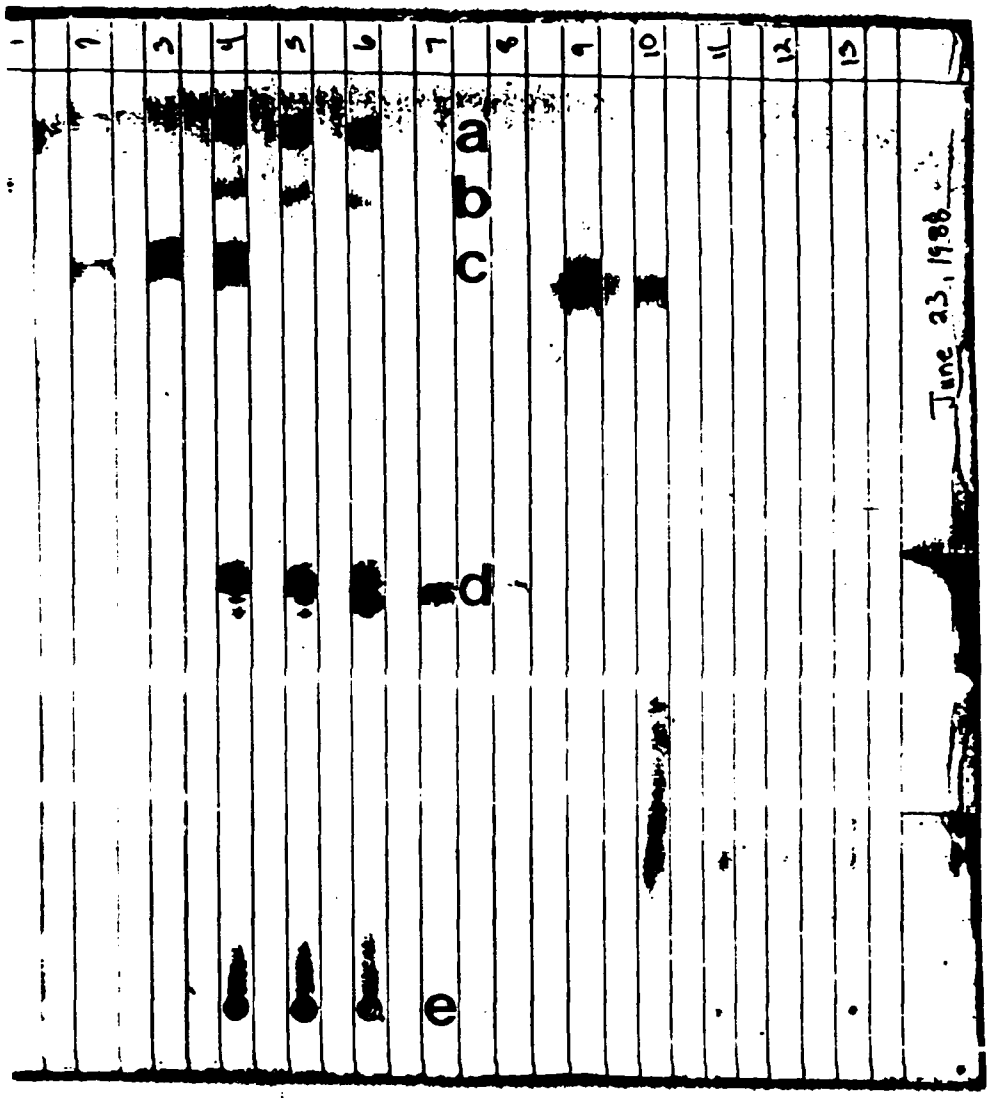


Figure 3

A--Cholesterol ester
B--Cholesterol
C--DHEA
D--DHEA-S
E--Origin

PREPRINT : JOURNAL MEDICAL VIROLOGY 26: --,1988.

PROTECTION AGAINST ACUTE LETHAL VIRAL INFECTIONS WITH THE NATIVE STEROID DEHYDROEPIANDROSTERONE (DHEA)

Roger M. Loria, Associate Professor Department of Microbiology, Immunology, and Pathology. Mr. Thomas H.Inge[1], Sallie S. Cook, Assistant Professor of Pathology and Medical Director of the Blood Bank, Andras K. Szakal, Associate Professor of Anatomy and William Regelson, Professor of Medicine. Schools of Basic Health Science and Medicine, Virginia Commonwealth University, Richmond Va. 23298.

[1]M.D./Ph.D. candidate.

Abbreviated title:

Protection Against Lethal Viral Infections

Key words:

Acute; Virus-Infection; Immune up-regulation; DHEA (dehydroepiandrosterone); coxsackievirus, herpesvirus.

Address all inquiries to Dr. Roger M. Loria, Department of Microbiology and Immunology, School of Basic Health Sciences, Virginia Commonwealth University, Medical College of Va. 23298-0678. Tel: (804) 786-9717.

ABSTRACT:

A significant protective effect of a native adrenal steroid, dehydroepiandrosterone (DHEA) was demonstrated in studies of two lethal viral infection models in mice, systemic coxsackievirus B4 and herpes simplex type 2 encephalitis. The steroid was active either by long-term feeding or by a single subcutaneous injection. A closely related steroid, etiocholanolone, was not protective in these models. Histopathological analysis, leukocyte counts, and numbers of spleen antibody forming cells in the coxsackievirus B4 model suggests that DHEA functions by maintaining or potentiating the immune competence of mice otherwise depressed by viral infection. DHEA was not effective in genetically immunodeficient HRS/J hr/hr mice and did not demonstrate antiviral activity in vitro. While the molecular basis for DHEA's effect on the immune system is not known, studies by others suggest that it may counteract the stress related immunosuppressive effects of glucocorticoids stimulated by viral infection. Because DHEA is a native steroid that has been used clinically with minimal side effects, the utility of DHEA in the therapeutic modulation of acute and chronic viral infections including acquired immune deficiency syndrome (e.g., HIV) deserves intensive study.

INTRODUCTION

Dehydroepiandrosterone, 3-beta-hydroxyandrost-5-en-17-one or dehydroiso-androsterone (DHEA), is quantitatively one of the major adrenal cortical steroid hormones in humans and other mammals [Windholz,1976; Diem,1975]. DHEA is sulfated by an adrenal sulfokinase to DHEA sulfate (DHEAS) in humans, but to a lesser extent in rodents [Tyrell, 1983]. DHEAS is quantitatively the major secretory product of the human adrenal gland [Migeon,1957] and the levels of this hormone begin to decline in the second decade of life reaching 5% of the original level in the elderly [Barret-Connor,1986]. Although DHEA appears to serve as an intermediary in gonadal steroid synthesis the primary physiological function of DHEA is unclear.

Our previous studies [Loria, 1984;1986; Montgomery,1986] have shown that the diabetic mutation db+/db+, is also associated with an impaired immune response in the inbred C57BL/KsJ mouse, and this host is markedly more susceptible to coxsackievirus B4 (CVB4) infection. It is now recognized that diabetes mellitus in humans may be a virus mediated autoimmune reaction which may result in the destruction of the islet of Langerhans [Markhost,1987; Bottazzo, 1986]. Since dietary DHEA was reported to have an anti-diabetic effects [Coleman,1982; 1984; 1984b; 1985] in the diabetic mutant mouse, we examined whether the anti-diabetic effect of DHEA could be mediated in part by an effect on the immune response and/or on the pathogenicity of the enterovirus CVB4. Two acute virus infection models, with distinct replicative and pathogenic mechanisms were examined to determine the effects of DHEA on virus-mediated pathophysiology. The results show that peroral (p.o) and subcutaneous (s.c.) administration of DHEA up-regulates the host immune system and reduces the virulence of an RNA and DNA virus that are lethal by widely different mechanisms.

MATERIALS AND METHODS

Viruses and tissue culture procedures

Two different human virus isolates were used to challenge C57BL/6J inbred mice, one was the CVB4 Edwards strain and the second was herpes simplex type 2 (HSV2). Details on the passage history of CVB4 and tissue culture procedures have been published previously ([Loria,1976; 1984; 1986]. The HSV2 strain MS was obtained from the American Type Culture Collection (ATCC VR-540). This virus was grown and plaqued on Vero Cells. For staining HSV2 plaques a 1% crystal violet was used for 20 minutes, and then rinsed.

Animals

Male mice have been shown to be more susceptible than female mice to enterovirus infection [Berkovitch, 1965,1967] and the reverse is true for HSV2 susceptibility [Mogensen, 1977; Baker, 1978; Yirrell,1987]. Therefore male inbred C57BL/6J mice 6 to 8 weeks old (Jackson Laboratories, Bar Harbor, ME) were infected with CVB4 while female inbred mice of the same age and strain were used for HSV2 infections. The genetically immunodeficient hairless female HRS/J hr/hr inbred mice (Jackson Laboratories, Bar Harbor, ME) at 6-8 weeks of age were used to test the effect of a functional immune system [Heiniger, 1974; Johnson, 1982] on the response to DHEA.

Diet

All animals were maintained on normal laboratory mouse chow Agway RMH-3000 (Agway, Syracuse, NY). In experiments were animals were maintained on a semipurified diet high in animal fat the diet contained 20% casein, 52.5% sucrose, 18% animal fat (lard), 5% cellulose, 4% salts, 0.2% choline chloride, 0.1% inositol and 0.1% vitamin mix. This semipurified diet has been used extensively [Loria, 1976a;1976b; Campbell,1978; 1982].

Route, Vehicle and dose

Several routes of DHEA administration were examined. These included feeding as 0.4% of the diet (p.o.), subcutaneous injection (s.c.) or intraperitoneal injection (i.p.). For injection, DHEA (Searle, Chicago IL.) was suspended in 0.2 ml dimethyl sulfoxide (DMSO). In CVB4 experiments, animals were infected with virus one hour after DHEA injection, and each group was challenged (i.p.) with a dose of CVB4 ranging from 10^2 pfu to 10^5 pfu/animal. In HSV2 infection experiments, young mice were challenged by intracranial (i.c.) injection with a dose of HSV2 ranging from 10^5 to 10^8 PFU/animal. Four hours prior to viral infection animals were injected s.c. with 1 g/kg of DHEA. Control animals were injected with virus, and 0.2 ml of DMSO.

The optimal dose of DHEA mediating antiviral activity was determined by injecting animals with DHEA doses of 2g, 1g, 500 mg and 250 mg/kg, respectively. Protection from lethal CVB4 and HSV2 infection was observed when DHEA was injected s.c. at a dose of 1g/kg. Also, feeding DHEA at 0.4% concentration protected from lethal virus infection. No significant protection from lethal virus infection was evident with any other s.c. dose of DHEA, or with any dose of etiocholanolone, 3 α-hydroxy-5β-androstan-17-one (Sigma Chemical Co. St Louis Mo.). In all subsequent experiments a dose of 1g/kg DHEA s.c. (25 mg/mouse) was used.

Enumeration of Spleen antibody forming cells

As previously described [Montgomery, 1986] ten days after CB4 infection test animals were subjected to an i.p. injection with 5 x 10^8 sheep red blood cells (SRBC), while control animals were immunized only with SRBC. Four days after SRBC immunization, animals were killed with an overdose of ether and the spleen removed. The procedure of Moller et al 1973, for the enumeration of spleen cells secreting IgM - antibody was used in these experiments.

Peripheral white blood cell counts

Peripheral white blood cells were counted following Diff - Quik (American Scientific Products, McGaw Park, IL.) staining of blood smear. No differentiation of lymphocytes or monocytes by special stains or cell marker was done.

Histopathological examinations

For histopathological studies animals were sacrificed by an overdose of methoxyflurane (Metofane, Pitman-Moore, Inc. Washington Crossing, NJ.) tissues were removed and fixed in phosphate buffered formaldehyde at room temperature. Specimens were embedded in paraffin, section and stained with hematoxylin and eosin.

Statistical Analysis

The General Linear Models Procedure (SAS) was used to determined the significance of the particular changes in a given cell type. Whether there was a significant difference between the various groups was determine using Tukey's studentized range test for each variable at a $p \leq 0.05$ level. A confirmation of these results was obtained from the non parametric Wilcoxon Rank Sums test.

RESULTS

Protection against lethal virus infection

The effects of 25 mg DHEA injected s.c. on the percent survival following CVB4 and HSV-2 infection are presented in Figures 1(a) and 1(b), respectively. The results show that the percent cumulative survival of animals following CVB4 infection was close to 90% in DHEA-treated mice as compared to about 58% in control animals ($p \leq 0.03$), Figure 1(a). An almost identical increase in the percent cumulative survival was evident in HSV2-infected and DHEA-treated mice 92% versus 58% in control HSV2-infected animals, Figure 1(b). The effects of s.c. injected DHEA on virus-dose dependent mortality following infection with CVB4 and HSV2 are presented in Figure 2(a) and 2(b). The findings show that animals infected with CVB4 LD_{100} dose (10^5 PFU/animal) had mortality reduced from 90 % to 37.5% with DHEA treatment, while HSV2 induced mortality was reduced from 88% to 0 at a dose of 10^7 pfu/animal . This protective effect of DHEA against i.p. CVB4 and intracranial HSV2 infections was statistically significant, $p \leq 0.03$. These results confirmed and extended our earlier observations which showed that inbred C57BL/6J mice fed 0.4% DHEA in a semi-purified diet high in animal fat for 16 weeks prior to challenge was also associated with a significant resistance to CVB4 infection, Figure 3, $p \geq 0.05$.

In these experiments, we also tested the effects of the sulfated metabolite, DHEAS, by s.c. or i.p. injection as well as the effects of the related steroid, etiocholanolone at the above mentioned doses. There was no evidence of protection against virus lethality with either DHEAS or etiocholanolone.

Number of spleen antibody forming cells

The effect of DHEA on the number of spleen antibody forming cells (AFC) in virus-infected and uninfected animals was determined by sheep red cell immunization as described previously. The number of IgM AFC per 10^6 spleen cells in uninfected and CVB4-infected mice with and without DHEA treatment are presented in Figure 4(a). As can be seen, the number of IgM AFC per 10^6 spleen cells was 35% higher in uninfected DHEA-treated mice as compared to the uninfected control mice. This increase was not statistically significant. However, in CVB4-infected DHEA-treated mice the number of spleen IgM AFC was 80% higher than the number of IgM AFC in CVB4-infected control mice, $p \leq 0.025$. The number of spleen IgG AFC were also enumerated (Figure 4b); in DHEA-treated/CVB4-infected mice a 70% increase in the number of IgG AFC are observed as compared to virus-infected mice not treated with DHEA. This increase however, was not statistically significant.

Histopathological examination

Histopathological studies of hematoxylin and eosin stained spleen sections revealed that the spleen peri-arteriolar sheath of lymphocytes (PALS), which is composed largely of T lymphocytes, that are primarily Thy 1.2 cells, were well developed in both DHEA-treated and in CVB4-infected animals. However, infection with CVB4 was associated with reduction in the number and size of spleen germinal centers. In contrast, in DHEA-treated animals, there was a marked increase in splenic germinal center development, suggesting B lymphocyte hyperplasia and a marked increase in the hematopoietic activity in the spleen red pulp areas. Furthermore, in untreated CVB4-infected animals, a prominent picture in the spleen was the "starry sky pattern" which is indicative of probable viral related cell killing. The "starry sky" morphologic pattern is produced by the prominence of activated macrophages amidst dense collections of lymphocytes and normal splenic vascularity [Sorger,1987; Sinkovics,1969]. In DHEA-treated/CVB4-infected animals, the "starry sky" pathological picture was reduced. These histological observations are suggestive of DHEA-mediated changes in the splenic lymphocyte and the hematopoietic cell populations.

Peripheral leukocyte counts

We also evaluated the effect of DHEA on peripheral leukocyte concentrations in the following four groups: [1] control ; [2] 1 g/kg DHEA s.c.; [3] CVB4-infected and [4] 1 g/kg DHEA s.c./ CVB4-infected. All data were analyzed for statistical significance and the results are presented in TABLE 1.

There was no significant effect of DHEA alone on the total leukocyte count as compared to untreated control animals. However, the total leukocyte count three days after infection was significantly lower in the DHEA-treated/CVB4-infected animals, as compared to uninfected control or DHEA injected controls, $p \leq 0.05$. There were no significant differences in the total leukocyte counts between any of the experimental groups at subsequent sampling. In contrast, three days after CVB4 infection only, or in the DHEA-treated/CVB4-infected group, the monocyte counts were 50% and 84.5% lower than the control group, respectively, $p \leq 0.05$. in contrast, seven days after infection the monocyte counts of the DHEA-treated CVB4-infected group were 214% higher than the monocyte counts in the group infected with CVB4 that did not receive DHEA, $p \leq 0.05$. DHEA injection alone without CVB4 infection resulted in a 62% elevation of monocyte counts over control animals. There was no significant difference in the monocyte counts between the CVB4-infected animals not receiving DHEA and uninfected controls.

A biphasic response in peripherally sampled

polymorphonuclear leukocyte (PMN) numbers was evident in CVB4-infected animals. Three days after infection the PMN counts reached 3.94×10^3 cells /mm^3 which was 515% higher than the PMN count in the control group of 0.64×10^3 cell/ mm^3, $p \leq 0.05$. A second elevation in PMN counts was seen at 14 days in CVB4-infected or DHEA-treated/CVB4-infected animals only. This elevation was not quite as accentuated and not statistically significant. In non-infected DHEA-treated animals no real change was noted in the number of PMNs.

Host immunogenetics

The mutation hairless hr/hr in the inbred HRS/J mouse is associated with hereditary immunodeficiency and leukemogenesis [Heiniger, 1974; Johnson, 1982; Holmes,1982]. Experiments were done to test whether s.c. DHEA injection could affect the resistance of this strain to CVB4 infection. Inbred HRS/J hr/hr mice were injected s.c. with 1 g/kg DHEA and challenged i.p. with 10^5 pfu/animal of CVB4 one hour later. In contrast to the immunologically normal inbred C57BL/6J mice, DHEA did not protect this immunodeficient mutant from CVB4 lethality.

Mode of DHEA administration

In contrast to protective s.c. DHEA injection our initial results show that DHEA given by the i.p. route was not associated with host protection from virus induced mortality or up - regulation of the immune response. We observed that s.c. injection of DHEA is associated with the formation of a local deposit leading to a probable prolonged DHEA interaction with the lymphoid system. Prolonged feeding of 0.4% DHEA was also protective in the CVB4 model, Figure 3. However, it is of particular interest to note that the magnitude and the range of protection against lethal virus infection associate with s.c. injection of DHEA was considerably greater than when DHEA was fed in the diet.

In vitro effect of DHEA

<u>In vitro</u> experiments were done to determine whether DHEA had any direct effect on CVB4 infectivity and replication. HeLa cells in culture were incubated with either 2 μM or 20 μM DHEA and inoculated with 100 pfu of CVB4. No evidence of a reduction in the number of CVB4 plaque forming units could be detected at these concentrations of DHEA.

DISCUSSION

In general, steroid hormones of adrenocortical origin when administered at pharmacological doses have been regarded as immunosuppressive [Cupps,1982; Claman, 1984; Grosmann,1984; Goldien, 1987; Parillo,1979], particularly in viral infections [Kilbourne, 1951; Gaitmaitan, 1970; Rytel,1969]. In viral infections the administration of glucocorticoids results in higher viral tissue titers and increased symptomatology [Lynden, 1984; Meek, 1972; Hollinger,1985; Johnson, 1985; Yirrell,1987].

In contrast, the results of this study demonstrate that DHEA, a native adrenal steroid hormone which has been thought to be primarily an intermediary in testosterone and estradiol metabolism [Tyrell, 1983], can prevent mortality normally seen with two distinct classes of viruses.

We are inclined to attribute the protection against viral lethality seen with a single s.c. injection of DHEA (but not DHEAS) to an effect upon the host resistance and/or the immune system rather than upon the viruses per see. This supposition is supported by the observations that [1] DHEA failed to influence CVB4 replication in vitro, where immune mechanisms are not present; [2] DHEA was ineffective in the inbred HRS/J hr/hr mouse which is genetically immunodeficient; [3] Up-regulation of the immune response by DHEA was seen in CVB4-infected mice with regard to the number of spleen IgM and IgG AFC, Figure 4; [4] Administration of DHEA alone was also associated with enlargement of the spleen germinal centers which suggests stimulation of the B lymphocyte dependent areas; [5] DHEA treatment of CVB4-infected animals resulted in a reduction of the "starry sky pattern", an indicator of cell killing, which was prominent in the spleens of CVB4-infected mice not treated with DHEA. [6] Finally, an increase in circulating mononuclear cells was observed in DHEA-treated/CVB4-infected mice which is consistent with the role of these cells in host defense against CVB4 infection [Woodruff,1979] as does the DHEA mediated decline in the splenic "starry sky" pattern.

While our studies do not reveal the specific effect(s) of DHEA on the immune system, there are suggestions from the work of [Riley, 1983] that DHEA may interfere with the immunosuppressive action of glucocorticoids such as corticosterone. In Riley's studies, mice subjected to "rotation stress" experienced increased serum corticosteroid levels and developed thymic involution and reduced resistance to transplantable tumors. These involutional effects of stress were antagonized by the s.c. injection of 1 mg/animal of DHEA [Riley, 1982]. In addition, DHEA also antagonized the effects of corticosterone injections on thymus involution.

Viral infections have been shown to cause an increase in glucocorticoid responses [Smith, 1982; Dunn,1987; Blalock, 1987; Hammond, 1972; Spackman, 1974; Santisteban, 1972] and thymic involution as well as a generalized immunosuppression [Escobar,

1983; Woodruff, 1975; Rager-Zisman, 1973; Thong, 1975]. Thus it is reasonable to speculate that DHEA or its metabolites could act to protect the immune system from the stressful effects of the infection, i.e. glucocorticoid-mediated immune suppression, and thus enhance the ability of the host to control virus mediated cytotoxicity, and possibly virus replication through various immune mechanisms. In this regard, a potent blocker of glucocorticoid synthesis, metyrapone, protects chickens against the lethal effects of Marek's disease, a herpesvirus-mediated lymphoproliferative disorder and also protect mice against murine sarcoma virus [Thompson, 1980; Rettura, 1973; Spangelo,1987]. Presently, the effects of exogenous DHEA on glucocorticoid synthesis and action are unknown. Similarly, it is not known whether DHEA can antagonize glucocorticoid action on T lymphocytes or other lymphoid cells. Both of these potential mechanisms of DHEA regulation of the immune system need to be investigated.

Since DHEA is considered to be a weak androgen its host protective antiviral effect must be examined in the context of known sex hormone effect on the immune system. In particular, estradiol and progesterone have been reported to have a effects on the natural killer cells [Grossman, 1985; Mohammad, 1985; Berci, 1986]. Thus DHEA like other gonadal and pituitary hormones [Davila, 1987; Russell 1985] could have an independent regulatory effects on the immune response.

An alternative, explanation for the sparing effect of DHEA in these acute viral infection models, is that this steroid hormone may reduce virus mediated T lymphocyte killing and reduce the number of anti-viral cytotoxic T cells, leading to a reduced tissue pathology. Indeed, cytotoxic T lymphocytes have a major role in the pathogenesis of CVB4 infection while humoral immunity is protective [Woodruff, 1975; 1979; Escobar, 1983; Rager-Zisman, 1973; Thong, 1975]. The opposite is seen in primary HSV infections [Lopez, 1984; Rouse, 1984] resistance is primarily mediated by T lymphocytes while antibody protection is not as significant. Our observation of an increased proliferation of the spleen germinal centers in DHEA-treated animals and the reduction in the viral killing of lymphocytes in DHEA-treated/ CVB4-infected animals support this hypothesis. Furthermore, the alteration of circulating leukocytes and the elevation in monocytes seen in DHEA-treated/CVB4-infected mice suggest a modulatory effect of DHEA on monocytes at various stages of the infection process. Monocytes have been reported to serve as effector cells in CVB4 infections [Woodruff, 1979] and it is possible that the changes in circulating monocyte levels reflect on the action of DHEA on tissue distribution or generation of these cells.

In our studies, anti-viral effects were observed only when DHEA was given s.c. or p.o., indicating that the route of DHEA administration may be a critical factor in the up-regulation of the host immune response. As is evident from figures 2 and 3, the magnitude and the range of protection against lethal virus

infection associated with s.c. injection of DHEA were considerably greater than when DHEA was fed in the diet. Recent reports show that the skin may have unique immune functions [Romerdahl,1986; Streilein, 1983]. Indeed the skin is known to contain a population of cutaneous immune cells, which include the epidermal Langerhans cells and keratinocytes which produce an epidermal thymocyte-activating factor, similar to IL-1 [Sauder,1984]; and in the murine system the Thy-1+ dendritic epidermal cell. It has been suggested that the Thy-1+ cell has a role in immune surveillance [Bergstresser, 1983; Tschachler, 1983] or in the presentation of antigen [Sullivan,1985]. Consequently, it is possible that the increase in resistance following s.c. DHEA injection is associated with activation of the skin particular immune functions.

The ability of DHEA to influence the immune system is also supported by the reports that DHEA and its bromo derivative have inhibited lymphoblastic transformation in human lymphocytes in vitro. In addition, DHEA has prevented the autoimmune lupus like syndrome in the NZB mouse that is thought by some to be caused by a slow virus [Henderson, 1981; Schwartz, 1985].

Whatever the mechanism of DHEA's action in the acute viral models, our studies suggest that prolonged exposure to DHEA is also an important factor for obtaining the protective effect. Either prolonged feeding for 16 weeks or s.c. deposition of the hormone appeared to be required for achieving antiviral protection (Figure 3), while i.p. bolus administration did not protect the host against CVB4 infection. Furthermore, injection of DHEA sulfate in the mouse, either s.c. or i.p. showed no anti-viral action, suggesting that the protective action of DHEA is through a pathway independent of sulfation.

An unexpected finding in these studies was that the protection seen with DHEA was enhanced by increasing the virus dose in both infection models, Figures 2(a) and (b). These results suggest that a certain critical virus load is required to activate the protective mechanism(s) induced by the hormone. This phenomenon could be mediated by the need for a certain amount of viral antigen to trigger the pertinent DHEA-modulated immunological process. Another possibility is that a threshold amount of virus might be required to activate the adrenal cortex if the protective DHEA effect should prove to be mediated through antagonism of glucocorticoids or other steroid effects.

The protective effect of s.c. DHEA injection against intracranial HSV2, Figures 1 and 2, was obtained by injection of the hormone four hours prior to infection. However, dose timing is critical, if injection of HSV2 i.c. and DHEA s.c. was one hour apart, no anti-viral effect was produced. This observation suggests that either DHEA has to penetrate the blood-brain barrier to achieve its effect or the lag is necessary for DHEA to achieve up-regulation of the host immune system.

Clinically, DHEA has been used systemically and/or topically for psoriasis, and has been used in the treatment of gout, hyperlipemia and in post coronary patients [Regelson, 1988]. In

animal models [Yen,1977] and humans it has anti-obesity effects [Nestler, 1988] and anti-carcinogenic action in animals [Lopez, 1984; Rouse, 1984; Henderson, 1981; Schwartz, 1985]. It is still used clinically in Europe in conjunction with estrogen as an agent to reverse menopausal systems and has also been used in the treatment of manic depression, schizophrenia and Alzheimer's disease. Our group has studied DHEA clinically at 40 mg/kg /day in the treatment of advanced cancer and we are involved in an ongoing study of its role in multiple sclerosis [Regelson, 1988]. Mild androgenic effects, hirsutism and increased libido, were the side effects observed.

Our results show that dietary and s.c. administration of DHEA provides a new, effective approach to the treatment of both RNA and DNA viral infection; it may have broad clinical value where immunosuppression is a manifestation of infectious pathology or aging. DHEA,in contrast to clinical corticosteroids, is not diabetogenic nor anti-inflammatory. Its benign clinical side effects [Regelson, 1988; Nestler, 1988] suggest that it may have a place in the clinical treatment of viral infections where immunosuppression is an important concomitant of the infectious process.

ACKNOWLEDGEMENT:

The technical assistance of Ms. Karen Cameron and Ms. Christine K. Hogan is recognized.

This work was supported in part by the Office of Naval Research (ONR) Contract N00014-85-K-0525, the National Foundation for Cancer Research, and The Enviroscience Foundation. The authors are indebted to Dr. Jeannine Majde of ONR for her guidance and assistance with this manuscript.

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TABLE 1

TREATMENT	DAY	TOTAL LEUKOCYTES	MONOCYTES	NEUTROPHILES	N
None	-1	11.20 ± 0.74	0.55 ± 0.13	1.04 ± 0.17	10
CONTROL	3	11.30[a] ± 1.87	0.84 ± 0.27	0.64 ± 0.16	3
DHEA	3	8.37 ± 1.04	0.58 ± 0.07	0.74 ± 0.12	6
CVB4	3	7.98 ± 0.53	0.42[c] ± 0.08	3.94[c] ± 1.00	4
DHEA + CVB4	3	3.94[b] ± 0.88	0.13[c,d] ± 0.02	2.60[b] ± 0.30	12
CONTROL	7	10.30 ± 1.84	0.58 ± 0.22	0.94 ± 0.33	3
DHEA	7	12.30 ± 1.30	0.94 ± 0.11	1.40 ± 0.25	6
CVB4	7	5.08 ± 1.02	0.44 ± 0.16	1.44 ± 0.46	6
DHEA + CVB4	7	8.13 ± 0.61	1.38[e] ± 0.29	2.16[d] ± 0.29	12
CONTROL	14	9.73 ± 0.49	0.31 ± 0.14	0.73 ± 0.21	3
DHEA	14	14.20 ± 1.92	0.50 ± 0.22	1.84 ± 0.17	5
CVB4	14	11.10 ± 2.35	0.67 ± 0.41	4.95 ± 2.43	2
DHEA + CVB4	14	14.00 ± 1.50	0.87 ± 0.15	4.73 ± 1.12	7

Table 1 : Legend

All values are mean cells count x $10^3/mm^3$ blood ± S.E.

[a] Control animals were injected with both vehicle and medium at the respective sites.

Based on analysis of variance (ANOVA) the overall change on the third day were statistically significantly different for the total leukocyte, monocyte, and neutrophils counts at $p \leq 0.002$, $p \leq 0.0001$ and 0.0003, respectively. On day 7, the change in monocytes count we statistically significant, $p \leq 0.02$.

Tukey's studentized range test for multiple comparison at a level of ≤ 0.05 was used to determined whether the difference between the particular groups was significant.

[b] different from uninfected control and DHEA-treated groups

[c] different from uninfected control.

[d] different from DHEA-treated uninfected group.

[e] different from CVB4-infected group.

Figure 1

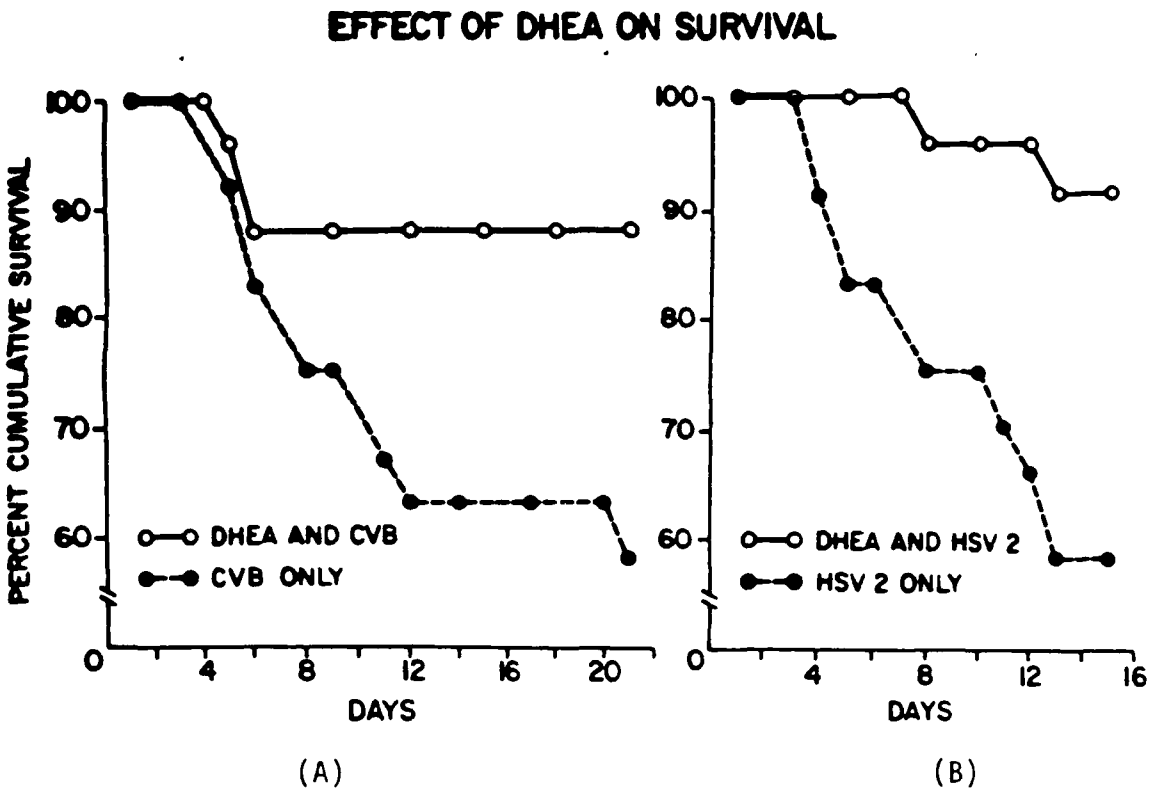


Figure 2

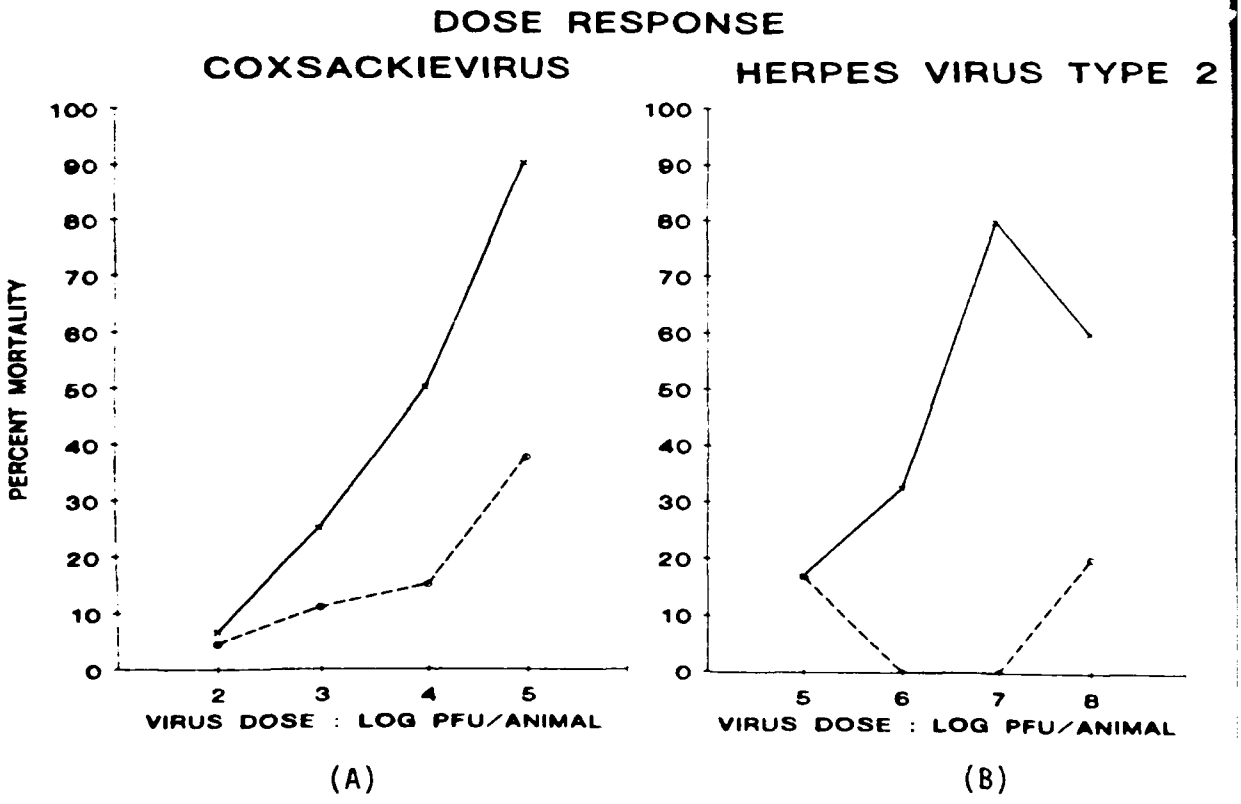


Figure 3

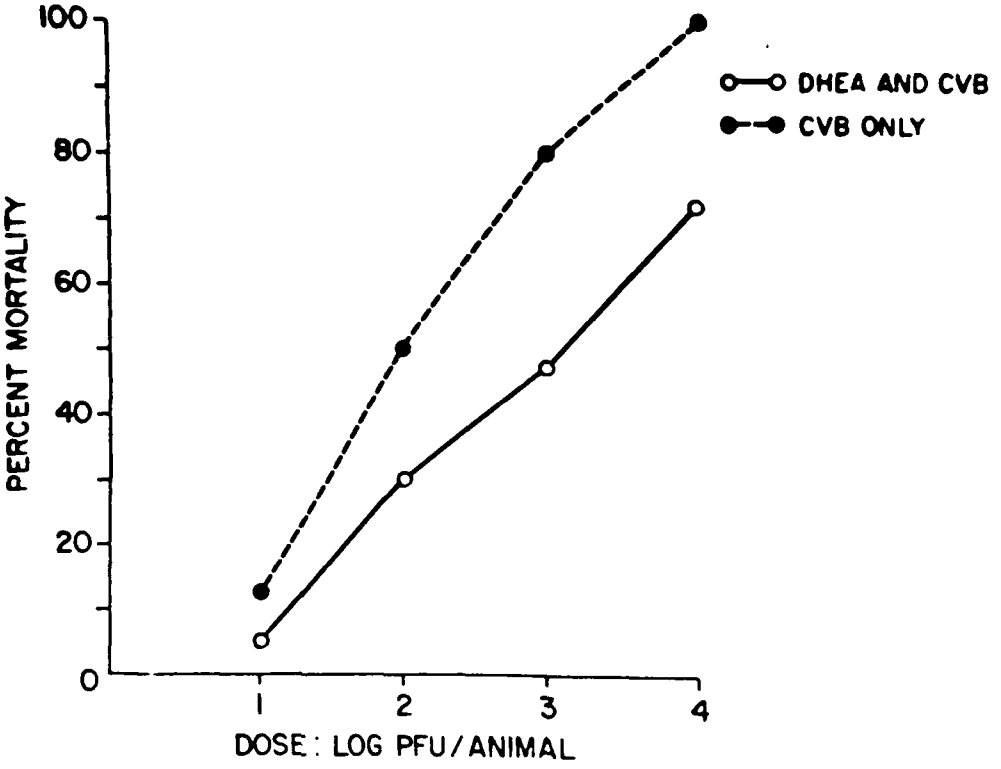
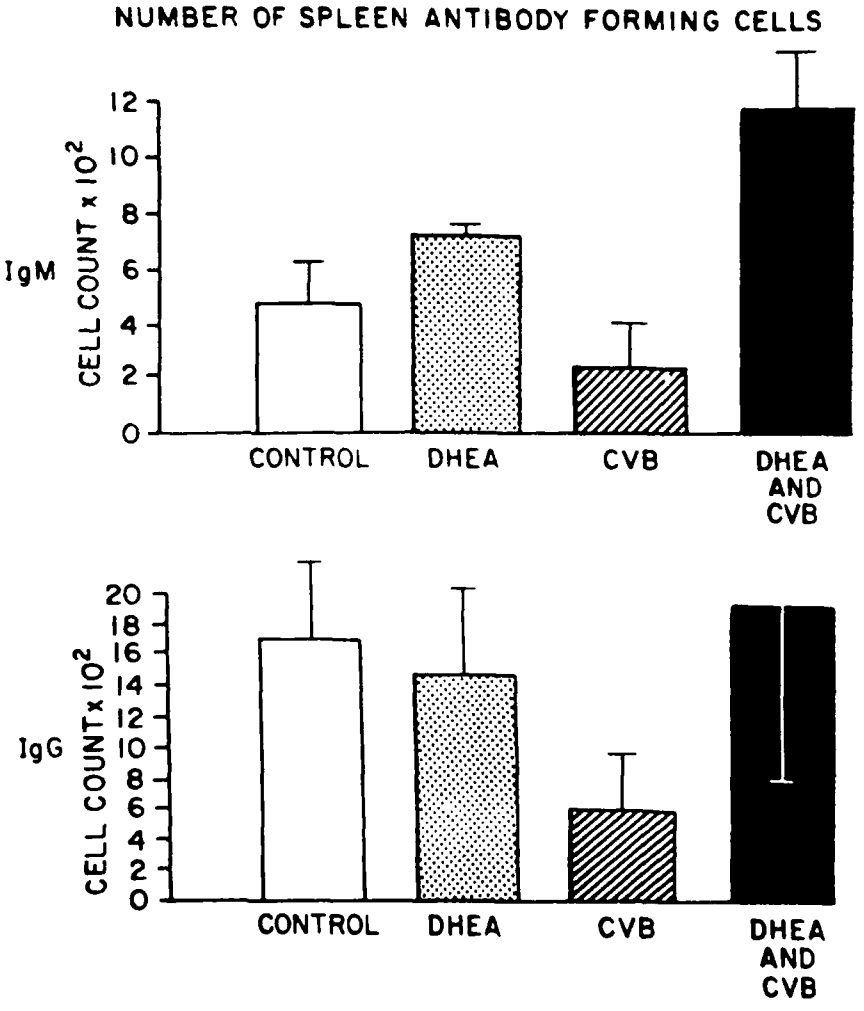


Figure 4



ABSTRACT FORM

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Up Modulation of the Host Immune Response for Anti-Viral Treatment with DHEA.

R.M. Loria, T.H. Inge and W. Regelson. Virginia Commonwealth University Schools of Basic Health Sciences and Medicine, Richmond Va. 23298

The results show that the steroid hormone dehydroepiandrosterone (DHEA) may have a significant role as an up modulator of the immune response, in contrast to the down regulation (immunosuppression) of other steroid hormones. The up regulation of the immune response was particularly evident when animals were infected with either an RNA or DNA virus. A subcutaneous injection of DHEA into male mice resulted in a significant protection from mortality induced by a human coxsackievirus B4. In virus infected animals only, administration of DHEA was associated with an 80% elevation of IgM and IgG antibody forming cells. Similarly, there was an 214% elevation in monocyte counts, again only in coxsackievirus infected and DHEA treated animals. Subsequent experiments with a DNA virus, herpes type II, demonstrated that DHEA administration was also effective in reducing mortality when this virus was injected intracranially. Initial results show that DHEA did not affect virus titers in vitro, and consequently its antiviral effect appears to be an indirect effect. Our observations demonstrate that up modulation of the host immunity by DHEA is an effective approach for the treatment of viral infections. This approach has also some major advantages since it can be used with or without conventional antiviral chemotherapeutic treatments.

Technical assistance Ms. Karen Cameron and Ms. Christine K. Hogan

Presenter's Phone No: (804) 786-9717 Preference: _____ Oral ___x___ Poster

Deadline: Completed abstract form and a self-addressed envelope must be received no later than January 11, 1988.

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ANTIVIRAL CONFERENCE
c/o Southern Research Institute
Post Office Box 55305
Birmingham, AL 35255-5305 USA
ATTN: Craig Battles

WORKSHOP PREFERENCE (Circle)

1. Drug synthesis & in vitro evaluation of new antiviral agents
2. Drug mechanisms of action
3. Utilization of molecular biology for drug targeting
4. Viral resistance
5. Immunotherapy
6. Animal models
7. Pharmacology (biological & clinical)
8. Clinical trials